Studies on Hindered Phenols and Analogues. 2. 1,3-Benzoxathioles Having SRS-A Inhibiting Activity

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A series of hindered phenolic 1,3-benzoxathioles (**7a**–l) were prepared and investigated for biological properties. Many compounds had LPO-lowering, antisuperoxide inhibiting, SRS-A inhibiting, and 5-lipoxygenase inhibiting activities. Among them, 5-hydroxy-4,6,7-trimethyl-2-propyl-1,3-benzoxathiole (**7d**) and 3-(5-hydroxy-4,6,7-trimethyl-1,3-benzoxathiol-2-yl)propanol (**7j**) were most potent in SRS-A inhibiting and 5-lipoxygenase inhibiting activities, respectively, and were selected for further development as candidate drugs for the treatment of asthma.

Studies on hindered phenols (TH) have caused great development in the field of antioxidants for polymers.[1] It has been well-known that phenols (TH), e.g., 2,6-di-*tert*-butyl-4-methylphenol (BHT, **1**), play the role of scavengers for the ROO• radical as shown in Chart I, in which RH and X• are unsaturated hydrocarbons and initiators such as active oxygen species, respectively.

Also, in vivo studies have demonstrated that the active-oxygen species including ROO•, HO•, and $O_2^{\bullet-}$ cause angiopathy related to arteriosclerosis, diabetic complications, and many kinds of inflammation.[2] As attempts to treat such diseases, applications of hindered phenols such as 4,4′-(isopropylidenedithio)bis[2,6-di-*tert*-butylphenol] (probucol, **2**),[3] 5-[4-[(6-hydroxy-2,5,7,8-tetramethylchroman-2-yl)methoxy]benzyl]-2,4-thiazolidinedione (CS-045, **3**),[4] 3,5-di-*tert*-butyl-4-hydroxyphenylazole derivatives (**4**),[5] α-(3,5-di-*tert*-butyl-4-hydroxybenzylidene)-γ-butyrolactone (KME-4, **5**),[6] and 2,6-di-*tert*-butyl-4-(2′-thenoyl)phenol (R-830, **6**)[7] have been proposed. Compounds **2**, **3**, and **4**–**6** are hypolipidemic, hypoglycemic, and antiinflammatory agents, respectively (Chart II).

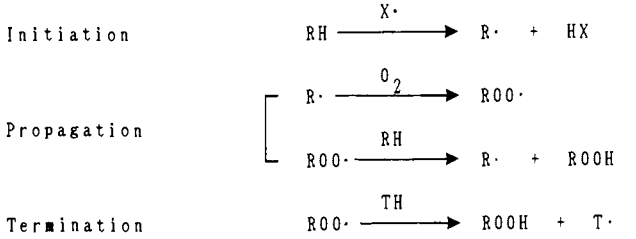
In a preceding paper,[4] we reported that compound **3** has hypoglycemic activity and lowers the level of lipid peroxide (LPO). During the course of this work, we found that hindered phenolic 1,3-benzoxathioles (**7**)[8] both lower LPO and inhibit the formation and release of the slow-reacting substance of anaphylaxis (SRS-A). In this paper, we wish to report on the synthesis and biological properties of 1,3-benzoxathioles.

Background of Drug Design

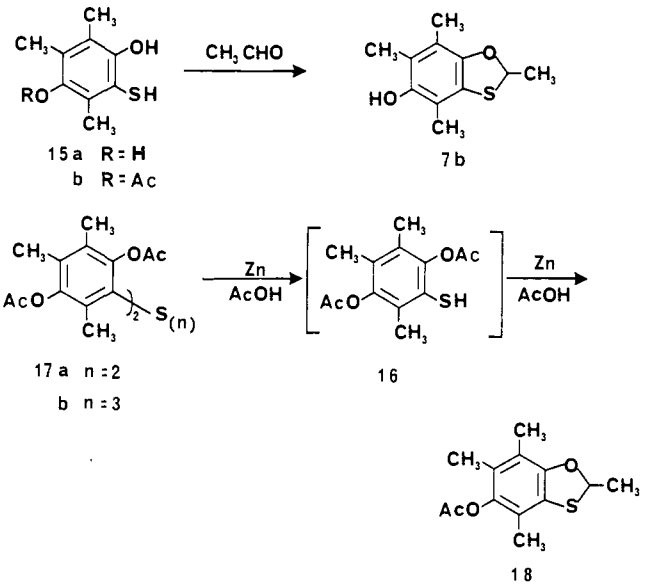
Angiopathy (such as arteriosclerosis), which is one of the most serious geriatric diseases, is caused by the rise in levels of blood lipid (cholesterol and/or triglyceride).[9] In recent years, it has been reported that such angiopathy is closely related to LPO.[10] We wish to find new compounds which lower both blood lipid and LPO.

In Chart III are shown well-known hypolipidemic agents ethyl 2-(4-chlorophenoxy)-2-methylpropionate (**8**, clofibrate)[11] and the corresponding sulfur analogue ethyl 2-[(3,5-di-*tert*-butyl-4-phenyl)thio]-2-methylpropionate (**9**).[12] The substructures **10**, **11**, and **12** in drugs **2**, **8**, and **9** gave us the idea of using substructure **13**, which seems not to have been used in the field of hypolipidemic[9] or hypoglycemic[13] agents (Chart IV). From the combination of **13** with the hindered phenolic group such as the 4-hydroxy-3,5,6-trimethylphenylene group (**14**), which is a substructure of vitamin E, a new type of hindered phenol, 5-hydroxy-1,3-benzoxathiole (**7**), was designed.

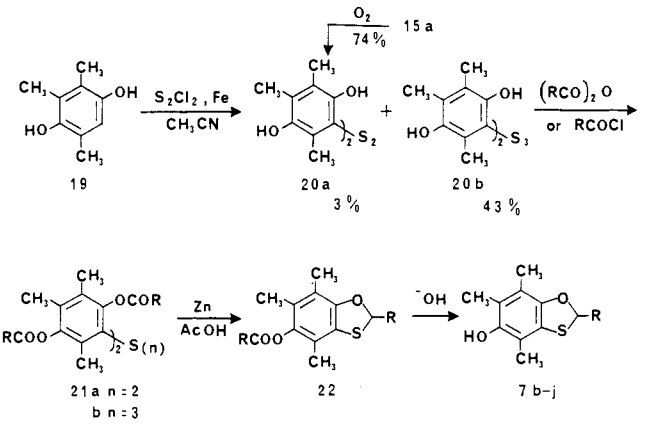
Chart I



Scheme I



Scheme II



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‡ Biological Research Laboratories.
§ Analytical and Metabolic Research Laboratories.

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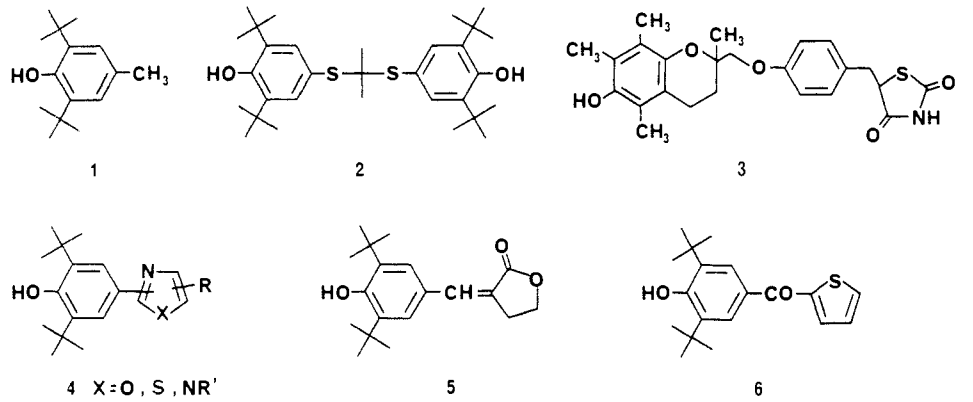
Chart II



Chemistry

2-Unsubstituted derivative (R = H) 5-hydroxy-4,6,7-trimethyl-1,3-benzoxathiole (**7a**) was prepared according to a reported method.[14a] 2-Methyl substituted (R = Me) **7b** must be prepared simply by the reaction of the corresponding mercaptohydroquinone (**15a**) with acetaldehyde in a manner similar to that described by Smith.[14b] However, the starting material **15** is sensitive to air oxidation (Scheme II) and therefore the handling of **15** may be somewhat troublesome. Furthermore, 2-mercaptophenol

Chart III

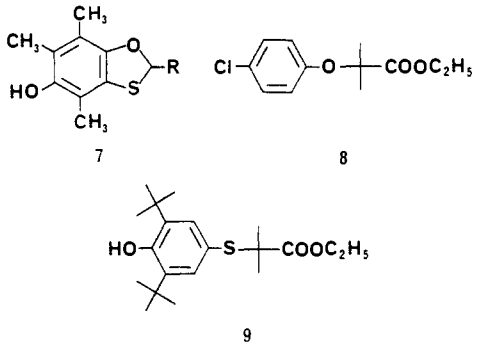


Chart IV

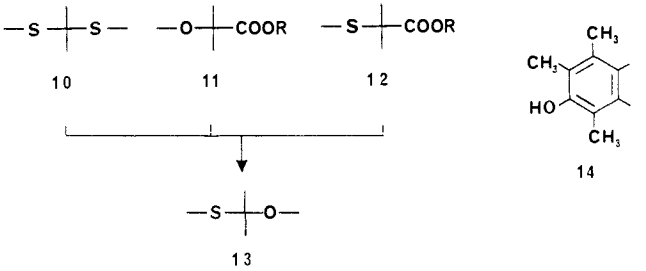
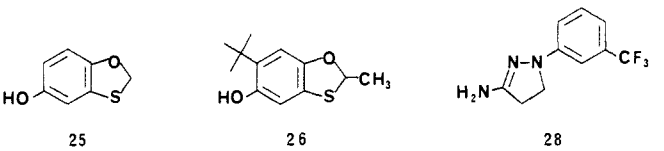
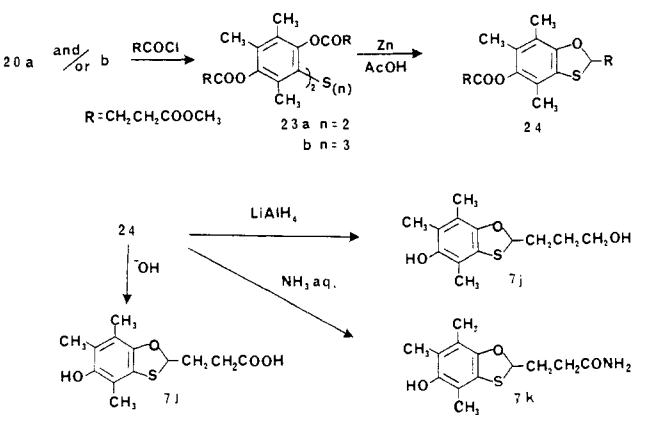


Chart V



Scheme III



(1) (a) Lundberg, W. O. *Autoxidation and Antioxidants*; Interscience: New York, 1961; Vol. 1, (b) Lundberg, W. O. *Autoxidation and Antioxidants*; Interscience: New York/London, 1962; Vol. 2.

(2) (a) Portera-Sanchez, A.; Calandre, L. *Atherosclerosis—Clinical Evaluation and Therapy*; Lenzi, S., Descovich, G. C., Eds.; MTP Press Ltd.: Lancaster, UK, 1982; Chapter 22. (b) Chait, A.; Heinecke, J. W.; Hiramatsu, K.; Baker, L.; Rosen, H. *Atherosclerosis VII*; Fidge, N. H., Nestel, P. J., Eds.; Elsevier Science Publishers B. V.: Amsterdam, 1986; p 491. (c) Asayama, K.; English, E.; Slonim, A. E.; Burr, I. M. *Diabetes* **1984**, *33*, 160. (d) Uchigata, Y.; Yamamoto, H.; Kawamura, A.; Okamoto, H. *J. Biol. Chem.* **1982**, *257*, 6084. (e) Oyanagui, Y. *Biochem. Pharmacol.* **1976**, *25*, 1465. (f) Salin, M. L.; McCord, J. M. *J. Clin. Invest.* **1975**, *56*, 1319.

(3) Neuworth, M. B.; Laufer, R. J.; Barnhart, J. W.; Sefranka, J. A.; McIntosh, D. D. *J. Med. Chem.* **1970**, *13*, 722.

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(5) Isomura, Y.; Sakamoto, S.; Ito, N.; Homma, H.; Abe, T.; Kubo, K. *Chem. Pharm. Bull.* **1984**, *32*, 152.

(6) (a) Katsumi, I.; Kondo, H.; Yamashita, K.; Hidaka, T.; Hosoe, K.; Yamashita, T.; Watanabe, K. *Chem. Pharm. Bull.* **1986**, *34*, 121. (b) Hidaka, T.; Hosoe, K.; Ariki, Y.; Takeo, K.; Yamashita, T.; Katsumi, I.; Kondo, H.; Yamashita, K.; Watanabe, K. *Jpn. J. Pharmacol.* **1984**, *36*, 77.

(7) Moore, G. G. I.; Swingle, K. F. *Agents Actions* **1982**, *12*, 674.

(8) Yoshioka, T.; Kitazawa, E.; Yamazaki, M.; Iizuka, Y. US Patent 4,691,027, 1987; *Chem. Abstr.* **1986**, *104*, 109610*r*.

(9) Fears, R. *Med. Actual.* **1984**, *20*, 257.

(10) Yagi, K. *Chiryo* **1979**, *61*, 867.

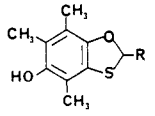
(11) Witiak, D. T.; Newman, H. A. I.; Feller, D. R. *Clofibrate and Related Analogs—A Comprehensive Review*; Marcell Dekker, Inc.: New York, 1977; p 1.

(12) Wagner, E. R.; Dull, R. G.; Mueller, L. G.; Allen, B. J.; Renzi, A. A.; Rytter, D. J.; Barnhart, J. W.; Byers, C. *J. Med. Chem.* **1977**, *20*, 1007.

(13) (a) Sarges, R. *Progress in Medicinal Chemistry*; Elsevier/North-Holland Biomedical Press: Amsterdam, 1983; Vol. 18, Chapter 6. (b) Steiner, K. E.; Lien, E. L. *Progress in Medicinal Chemistry*; Elsevier Science Publishers, B. V.: Amsterdam, 1987; Vol. 24, Chapter 6.

(14) (a) Degani, I.; Dolci, M.; Fochi, R. *Syn. Commun.* **1980**, *10*, 161. (b) Smith, G. E. P., Jr. US Patent 2,797,513; *Chem. Abstr.* **1961**, *55*, 21145*i*.

Table I. 1,3-Benzoxathioles



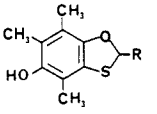
no.	R	mp, °C	formula	anal.
7a	H	131–132	$C_{10}H_{12}O_2S$	C, H, S
7b	Me	125–127	$C_{11}H_{14}O_2S$	C, H, S
7c	Et	85–85.5	$C_{12}H_{16}O_2S$	C, H, S
7d	Pr	72–73	$C_{13}H_{18}O_2S$	C, H, S
7e	Bu	73.5–74.5	$C_{14}H_{20}O_2S$	C, H, S
7f	C_6H_{13}	76.5–77	$C_{16}H_{24}O_2S$	C, H, S
7g	C_7H_{15}	76–76.5	$C_{17}H_{26}O_2S$	C, H, S
7h	n-$C_{19}H_{39}$	96–96.5	$C_{29}H_{50}O_2S$	C, H, S
7i	Ph	129–132	$C_{16}H_{16}O_2S$	C, H, S
7j	$(CH_2)_3OH$	116.5–118	$C_{13}H_{18}O_3S$	C, H, S
7k	$(CH_2)_2CONH_2$	160–161	$C_{13}H_{17}NO_3S$	C, H, N, S
7l	$(CH_2)_2COOH$	138–140	$C_{13}H_{16}O_4S$	C, H, S
25		76–78	$C_7H_6O_2S$	C, H, S
26		63.5–65	$C_{12}H_{16}O_2S$	C, H, S

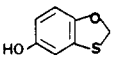
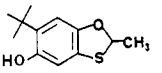
having a blocked hydroxyl group at the 4-position, e.g. 4-acetoxy-2-mercapto-3,5,6-trimethylphenol (**15b**), is somewhat troublesome to prepare. During the course of a synthetic investigation on preparing the 2-mercaptohydroquinones such as **15a**, **15b**, and 1,4-diacetoxy-2-mercapto-3,5,6-trimethylbenzene (**16**), we found by chance that reduction of 3,3′,5,5′,6,6′-hexamethyldithiobis(2,1,4-benzenetriyl) tetraacetate (**17a**) by zinc dust in acetic acid directly gave our desired 5-acetoxy-2,4,6,7-tetramethyl-1,3-benzoxathiole (**18**), instead of **16** (Scheme I). We further found that the corresponding trithio analogue (**17b**) gave also **18**. This ring closure occurs with the sulfur–sulfur bond cleavage followed by the participation of the neighboring acetoxyl group with the generated mercapto group of **16**. The formation mechanism of **18** is under investigation in detail. This method is useful for preparing the 1,3-benzoxathiole ring in general. The starting materials dithiobishydroquinones (**20a**) and trithiobishydroquinones (**20b**) were prepared as the minor and major products, respectively, by the reaction of trimethylhydroquinone (**19**) with sulfur monochloride in the presence of iron powder (Scheme II). In the scheme the substituents on the benzene ring are exemplified by a methyl group. Also, the oxidative dimerization of compound **15a** gave **20a** in 74% yield. Dithio and trithio derivatives **20a** and **20b** were acylated by acid anhydride or acyl chloride to give the corresponding tetrakis esters **21a** and **21b**, respectively. Both tetrakis esters **21a** and **21b** were reduced by zinc dust in acetic acid to give 5-(acyloxy)-2-substituted 4,6,7-trimethyl-1,3-benzoxathioles (**22**). Esters **22** were hydrolyzed to give hindered phenolic 1,3-benzoxathioles **7**. This method is able to use the preparation of 1,3-benzoxathiole substituted with an alkyl group containing a subgroup at the 2-position of the ring, e.g., 2-[(2-methoxycarbonyl)ethyl]-1,3-benzoxathiole (**24**). The 2-(methoxycarbonyl)ethyl group at the 2-position of the ring was introduced by using 3-(methoxycarbonyl)-propionyl chloride as the acylating agent. Other derivatives **7j–l** were prepared by LAH reduction, amidation, and hydrolysis of **24**, respectively (Scheme III).

Nonhindered 5-hydroxy-1,3-benzoxathiole (**25**) was prepared as in a reported method[14a] in order to examine the effect of hindered phenolic hydroxyl group in biological activities (Chart V).

6-*tert*-Butyl-2-methyl-1,3-benzoxathiole (**26**), which seems to have less hindered phenolic property, was prepared by the method described above by using *tert*-butylhydroquinone as the starting material (Chart V). The benzoxathioles synthesized are listed in Table I.

Table II. LPO Lowering and Antisuperoxide Activities of 1,3-Benzoxathioles



no.	R	LPO (IC_{50}, μg/mL)[a]	$Q_{1/2}$,[b] mM
7a	H	<0.1	1.3
7b	Me	<0.1	1.6
7c	Et	<0.1	1.3
7d	Pr	<0.1	1.4
7e	Bu	<0.1	1.6
7f	C_6H_{13}	0.1–0.3	1.6
7g	C_7H_{15}	<0.1	1.4
7h	n-$C_{19}H_{39}$	>1.0	1.8
7i	Ph	<0.1	2.4
7j	$(CH_2)_3OH$	0.1–0.3	2.2
7k	$(CH_2)_2CONH_2$	0.3–1.0	2.3
7l	$(CH_2)_2COOH$	>1.0	1.8
25		>1.0	6.2
26		0.1–0.3	
1		0.1–0.3	0.45
	vitamin E	>1.0[c]	4.4

[a] Rat liver microsomal lipid peroxidation. [b] Antisuperoxide activity. The concentration of the test compound required to inhibit generation of superoxide by 50%. Each value was the mean of three determination. [c] Acetate.

Results and Discussion

Hypolipidemic activity was investigated by a method using hyperlipidemic mice (C57BL/6NCrj strain)[15] fed a high-carloric diet. However, hypolipidemic activity was less potent than that of standard hindered phenolic compound **2** at oral doses up to 200 mg/kg (see the supplementary material).

LPO-lowering activity was estimated by the IC_{50} value of compounds in rat liver microsomal lipid peroxidation (Table II, LPO).[16] Standard compounds vitamin E and compounds **1** and **5** had IC_{50} values of >1.0, 0.1–0.3, and 0.1–0.3 μg/mL, respectively. Hindered phenolic 1,3-benzoxathioles having a hydrogen atom or alkyl groups at the 2-position were most potent and had IC_{50} values of <0.1 μg/mL, except hexyl (**7f**) and nonadecyl (**7h**). Other ones having alkyl groups substituted with a polar group such as a hydroxyl, carbamoyl, or carboxyl group were less potent (**7j–l**). Compounds **7j–l** showed that the increase of polarity results in a lowering of the activity.

Peroxidation of unsaturated fatty acid esters, degradation of polymers, and peroxidation in rat liver microsomes have been reported to be related to the superoxide radical.[1,17,18] Thus, antisuperoxide activity of **7** was fundamentally investigated by using a physical procedure as follows. The concentration of superoxide generated by an electrochemical procedure (see the Experimental Section)

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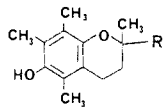
(18) Rotilio, G. Ed., *Superoxide and Superoxide Dismutase in Chemistry, Biology and Medicine*, Elsevier Science Publishers: Amsterdam, 1986.

Table III. LPO Lowering and Antisuperoxide Activities of Chromans



no.	R	LPO (IC_{50}, μg/mL)[a]	$Q_{1/2}$,[b] mM
27a	CH_3	<0.1	2.4
27b	CH_2OH	0.3–1.0	2.0
27c	COOH	>1.0	1.9

[a,b] See footnotes a and b, respectively, in Table II.

was measured by cyclic voltammetry. The concentration of the test compound required to inhibit generation of superoxide by 50% ($Q_{1/2}$, mM) was determined. Standard compounds vitamin E and compound 1 had $Q_{1/2}$ values of 4.4 and 0.45 mM, respectively. All of the 1,3-benzoxathioles showed greater activity ($Q_{1/2}$ < 2.5 mM) than vitamin E; 2-(lower alkyl substituted) derivatives (R = H, Me, Et, Pr, and Bu, **7a–e**) especially were very potent with $Q_{1/2}$'s ranging from 1.3 to 1.6 mM (Table II). Thus, 2-(lower alkyl substituted) derivatives (**7a–e**) were very potent in both LPO inhibiting and antisuperoxide activities. Nonhindered derivative **25** was less potent than hindered phenolic derivatives, especially than **7a–g**.

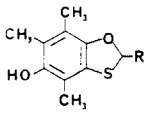
With the other groups beside the groups described above, the LPO-lowering activities were depressed by the polar group in the alkyl group at the 2-position of the 1,3-benzoxathiole ring (Table II). We took an interest as to whether or not this relationship applies in a series of chroman ring compounds. Table III shows LPO-lowering and antisuperoxide activities of 6-hydroxy-2,5,7,8-tetramethylchromans (**27a–c**) substituted by a methyl, hydroxymethyl, or carboxy group at the 2-position. These chroman compounds were synthesized by the procedure described in ref 19. In both ring systems, LPO-lowering activity depends on the polarity of the substituent; antisuperoxide activity was high and independent of the polarity of the substituent.

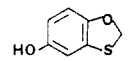
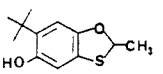
Some LPO-lowering agents have been reported to also have antiinflammatory activity or inhibiting activity on the formation and release of SRS-A as described before,[5-7] so we investigated the inhibiting activity of the 1,3-benzoxathioles on the formation and release of SRS-A. Table IV shows the IC_{50} value (μM), which was calculated on the basis of a regression line prepared from inhibition percents and each dose by the least-square method. Its confidence limits (95% CL) were calculated by Fieller's equation. The table shows that the hindered phenolic 1,3-benzoxathioles with a hydrogen atom or lower alkyl groups at the 2-position (**7a–d**) had activity higher than those with higher alkyl groups such as hexyl (**7f**) and heptyl (**7g**). Among **7j–l**, the most polar compound, 2-(3-carboxypropyl) derivative **71**, was inactive, similar to the case of the LPO-lowering activity. To investigate the contribution of the hindered phenolic property, the activities of nonhindered phenolic compound **25** and the less hindered one (**26**) were examined. The former was inactive (IC_{50} > 64.8) and the latter was less active (IC_{50} = 11.2). In passing, 3-amino-1-[3-(trifluoromethyl)phenyl]-2-pyrazoline (BW 755C, **28**), which is known as a 5-lipoxygenase inhibitor, and **5** were also investigated. Both showed extremely low activity.

SRS-A has been recognized to be a major bronchial-contracting substance relating to bronchial asthma.

(19) Scott, J. W.; Cort, W. M.; Harley, H.; Parrish, D. R.; Saucy, G. *J. Am. Oil Chem. Soc.* **1974**, *51*, 200.

Table IV. Inhibition of the Formation and Release of SRS-A and 5-Lipoxygenase by 1,3-Benzoxathioles



no.	R	IC_{50}, μM: SRS-A[a]	IC_{50}, μM: 5-lipoxygenase[b]
7a	H	2.4 (1.6–3.7)	0.29 (0.21–0.39)
7b	Me	2.1 (1.8–2.7)	0.13 (0.088–0.18)
7c	Et	4.5 (3.2–6.2)	0.063 (0.051–0.080)
7d	Pr	3.8 (2.9–5.0)	0.025 (0.017–0.035)
7e	Bu	11.9 (7.1–19.8)	0.059 (0.044–0.079)
7f	C_6H_{13}	>35.6	0.17 (0.11–0.24)
7g	C_7H_{15}	>33.9	0.22 (0.15–0.31)
7i	Ph	6.6 (4.0–10.3)	0.071 (0.057–0.090)
7j	$(CH_2)_3OH$	1.5 (1.0–2.0)	0.090 (0.072–0.114)
7k	$(CH_2)_2CONH_2$	3.1 (2.5–3.9)	0.52 (0.38–0.69)
71	$(CH_2)_2COOH$	>37.2	
25		>64.8	
26		11.2	
5		147	0.69 (0.45–0.99)
28		136	0.75 (0.60–0.95)

[a] SRS-A inhibiting activity (95% confidence limits). [b] 5-Lipoxygenase inhibiting activity (95% confidence limits).

Therefore, an antagonist of SRS-A or an inhibiting agent of formation or of release of SRS-A is expected to be effective for the treatment of asthma.[20] A compound having an inhibiting activity on 5-lipoxygenase is able to inhibit the formation of SRS-A, so we investigated the 5-lipoxygenase inhibiting activity of compound **7**. As shown in Table IV, 1,3-benzoxathioles had very strong activity in general. Among them, 2-propyl derivative **7d** was the most potent compound, with an IC_{50} value of 0.025 μM, and thus was 30 times more potent than compound **28**. Therefore, these compounds are postulated to inhibit the induction of SRS-A by inhibiting 5-lipoxygenase as a major factor.

Of newly synthesized hindered phenolic 1,3-benzoxathioles, most compounds had antisuperoxide activity, LPO-lowering activity, SRS-A inhibiting activity, and 5-lipoxygenase inhibiting activity. Among them, compounds **7d** and **7j** were most active in SRS-A-inhibiting and 5-lipoxygenase-inhibiting activities, respectively, and were selected for further development.

Experimental Section

Mass spectra were recorded on a JEOL-JMS-01SG or JEOL-JMS-D300 mass spectrometer. Infrared (IR) spectra were recorded on a IRA-2 infrared absorption spectrometer in Nujol mull. Proton magnetic resonance (NMR) spectra were recorded on a 90-MHz Varian EM-390 spectrometer and are reported in parts per million (δ) downfield from the internal standard tetramethylsilane (Me_4Si); the abbreviation "nd" means that precise identification of the signal was not possible because of overlap by other signals or absorption of solvent. All NMR spectra were consistent with the structures assigned. Column chromatography was performed on Merck-60 silica gel with the reported solvents. Melting points were determined on a Yanaco micro melting point apparatus and are uncorrected.

3,3′,5,5′,6,6′-Hexamethyl-2,2′-dithiobishydroquinone (20a) and 3,3′,5,5′,6,6′-Hexamethyl-2,2′-trithiobishydroquinone

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(20b). (a) Preparation of a Dithio Compound Using a Thiol Compound. A mixture of 7.5 g (40.7 mmol) of 2-mercapto-3,5,6-trimethylhydroquinone (**15a**) and 100 mL of Et_2O was allowed to stand in the air at room temperature for 1 day. The solvent was then evaporated, and the residue was recrystallized from THF to give 5.5 g (15 mmol) of **20a** as vivid yellow crystals, melting at 227–230 °C. IR ν_{max} (cm^{-1}): 3380, 3425.

(b) Preparation Using Sulfur Monochloride. Trimethylhydroquinone (**19**, 30 g, 0.197 mol) was dissolved in 1.2 L of anhydrous MeCN. Iron powder (1.4 g, 25 mg-atom) was then added to the solution. The solution was then cooled to −30 °C, and a solution of 13.3 g (0.0985 mol) of sulfur monochloride in 50 mL of anhydrous MeCN was added dropwise to the mixture under a nitrogen stream. The reaction temperature was then allowed to rise to room temperature, and the reaction mixture was stirred for 4 h. The precipitate produced was filtered off and the filtrate was condensed. The residue was purified by column chromatography. From the fraction eluted with a 10:0.5 (v/v) mixture of benzene and THF was obtained 1.0 g (2.7 mmol) of **20a**, which had the same melting point and IR spectrum as did the product of a.

From the precipitate which had been filtered off and was in the form of crystals was obtained 17.0 g (42.7 mmol) of **20b**, melting at 190–193 °C dec. IR ν_{max} (cm^{-1}): 3400.

General Procedure. 3,3′,5,5′,6,6′-Hexamethyltrithiobis-(2,1,4-benzenetriyl) Tetraacetate (17b). Compound **20b** (10 g, 25.1 mmol) was dissolved in 70 mL of pyridine. To the solution was added 30 g (0.294 mol) of Ac_2O, and the mixture was stirred for 24 h at room temperature. The reaction mixture was poured into water and the resulting precipitate was taken up in $CHCl_3$. The organic layer was separated, washed with water, and dried over Na_2SO_4. The solvent was distilled off and the residue was recrystallized from benzene to give 5.7 g (10.1 mmol) of **17b**, melting at 187–190 °C. NMR spectrum (δ ppm, $CDCl_3$): 2.08 (12 H, s), 2.32 (12 H, s), 2.41 (6 H, s). IR ν_{max} (cm^{-1}): 1760. MS (m/z): 566 (M^+).

5-Acetoxy-2,4,6,7-tetramethyl-1,3-benzoxathiole (18). (a) Preparation Using a Trithio Compound and Zinc Powder. Compound **17b** (6.3 g, 11.1 mmol) was dissolved in 120 mL of AcOH. To the solution was added 13 g (0.199 g-atom) of zinc powder, and the mixture was heated under reflux for 10 h under a nitrogen stream. The insolubles produced were filtered off, and the resulting solution was condensed to give a crude oil, which was dissolved in benzene. The benzene solution was washed with water and dried over Na_2SO_4. The solvent was distilled off and the resulting residue was purified by column chromatography. From the fraction eluted with benzene was obtained 4.4 g (17.4 mmol) of **18**, and this was recrystallized from hexane to give 3.5 g of a pure product, melting at 72–74 °C. NMR spectrum (δ ppm, $CDCl_3$): 1.76 (3 H, d, J = 6 Hz), 1.97 (3 H, s), 1.98 (3 H, s), 2.08 (3 H, s), 2.28 (3 H, s), 6.12 (1 H, q, J = 6 Hz). IR ν_{max} (cm^{-1}): 1750. MS (m/z): 252 (M^+).

(b) Preparation Using a Dithio Compound and Zinc Powder. The reaction, treatment, and purification of the reaction mixture described in a were repeated, but with **17a** instead of **17b**, to give **18**. The melting point and IR spectrum of this product were identical with those of the product obtained as described in a.

5-Hydroxy-2,4,6,7-tetramethyl-1,3-benzoxathiole (7b). Compound **18** (2.9 g, 11.5 mmol) was dissolved in 30 mL of MeOH. NaOMe (1.3 g, 24.1 mmol) was added to the solution, and the mixture was allowed to react at room temperature for 3 h. The reaction mixture was poured into water and the solution was neutralized by adding AcOH. The separated product was extracted with benzene and the extract was washed with water and dried over Na_2SO_4. The solvent was evaporated off. The crude product thus obtained was purified by column chromatography. From the fraction eluted with a 1:1 (v/v) mixture of hexane and benzene was obtained 2.0 g (9.51 mmol) of **7b**, melting at 125–127 °C. NMR spectrum (δ ppm, $CDCl_3$): 1.86 (3 H, d, J = 6 Hz, 2-CH_3), 6.45 (1 H, q, J = 6 Hz, 2-H). IR ν_{max} (cm^{-1}): 3330.

3-(5-Hydroxy-4,6,7-trimethyl-1,3-benzoxathiol-2-yl)-propanol (7j). Lithium aluminum hydride (10 g, 0.264 mol) was suspended in 500 mL of THF, and then a solution of 32.0 g (86.9 mmol) of **24** in 160 mL of THF was added dropwise with stirring and ice cooling under a nitrogen stream to the resulting suspension. After dropwise addition, the reaction mixture was stirred for 1 h at room temperature and then refluxed for 4 h. The reaction mixture was then cooled with ice–water, after which a mixture of 10 g of AcOEt and 50 mL of THF was added dropwise. The mixture was stirred for 1 h at room temperature, after which aqueous THF was added dropwise. The reaction mixture was then poured into ice–water. The pH of the mixture was adjusted to an acidic value by the addition of 10% HCl, and the precipitating product was extracted with AcOEt. The extract was washed with water and dried over Na_2SO_4. The solvent was distilled off under reduced pressure. The resulting crude product was subjected to column chromatography and **7j**, melting at 116.5–117 °C, was obtained from the fraction eluted with a 9:1 (v/v) mixture of benzene and AcOEt. NMR spectrum (δ ppm, $CDCl_3$): 1.52 (1 H, br s, disappeared with addition of D_2O, CH_2OH), 1.65–1.95 (2 H, m, $CH_2CH_2CH_2OH$), 1.95–2.3 (2 H, nd, $CH_2CH_2CH_2OH$), 3.6–3.9 (2 H, m, changed to 3.70 (2 H, t, J = 6 Hz) with addition of D_2O, CH_2OH), 6.05 (1 H, t, J = 6 Hz, 2-H). IR ν_{max} (cm^{-1}): 3360, 3100. MS (m/z): 254 (M^+).

3-(5-Hydroxy-4,6,7-trimethyl-1,3-benzoxathiol-2-yl)-propionamide (7k). Compound **24** (4.9 g, 13.3 mmol) was dissolved in 60 mL of MeOH, and 17 g of 28% w/v aqueous ammonia was added to the solution. The reaction mixture was then allowed to react for 5 days at room temperature. The solvent was distilled off under reduced pressure, and the residue was dissolved in AcOEt. The resulting solution was washed with water and dried over Na_2SO_4. The solvent was then distilled off under reduced pressure. The resulting crude product was subjected to column chromatography, and compound **7k**, melting at 160–161 °C, was obtained from the fraction eluted with a 3:7 (v/v) mixture of benzene and AcOEt. NMR spectrum (δ ppm, DMF-d_7): 2.15–2.6 (4 H, m, $-CH_2CH_2-$), 6.12 (1 H, t, J = 6 Hz, 2-H), 6.5–7.0 (1 H, br, disappeared with addition of D_2O, $CONH_2$), 7.7–8.1 (1 H, br, disappeared with addition of D_2O, $CONH_2$). MS (m/z): 267 (M^+).

Inhibition of Lipid Peroxide Formation. This was investigated by the ferrous sulfate/cysteine method described by Malvy et al.[16a] The test compound, cysteine (500 μM), and ferrous sulfate (5 μM) were added and allowed to react with rat liver microsomes. The amount of lipid peroxide thus formed was measured according to the thiobarbituric acid (TBA) method,[16b] and the concentration of the test compound required to inhibit the formation of lipid peroxide by 50% (IC_{50}, μg/mL) was estimated.

Antisuperoxide Activity. This activity was measured by a reported method.[21]

Inhibition of Formation and Release of SRS-A. The ability of the compounds to inhibit the formation and release of SRS-A was investigated by employing the technique of Watanabe-Kohno and Parker.[22] In these experiments, albumin was used as the antigen and was added to sensitized guinea pig (Hartley, male) lung slices 15 min after preincubation with the test compound. The amount of SRS-A formed and released under these conditions was assayed by a superfusion method, using a preparation of isolated guinea pig ileum. Other details of the technique employed are as described by Watanabe-Kohno and Parker. From the results was determined the concentration of the test compound required to inhibit by 50% formation and release of SRS-A (IC_{50}, μM). The results are shown in Table IV.

Inhibition of 5-Lipoxygenase. Preparation of Enzyme. The preparation of polymorphonuclear leukocytes (PMNL) was mostly based on the method of Sbarra and Karnovsky.[23] A 2% casein solution was injected intraperitoneally into guinea pigs weighing 400–500 g. After 14–16 h, the guinea pigs were sacrificed, and the peritoneal exudate was collected. The PMNL were harvested, washed once, and resuspended in 50 mM potassium phosphate buffer (pH 7.4) containing 10% ethylene glycol and 1 mM EDTA at a density of 2×10^7 cells/mL.[24] The cell sus-

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pension was sonicated at 20 KHz for 30 s twice, and the sonicate was centrifuged at 10000g for 10 min. The supernatant solution was stored at -80 °C.

Enzyme Assay. The activity of 5-lipoxygenase was assayed by a modification of the procedure of Ochi et al.[25] The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.4), 2 mM $CaCl_2$, 1 mM glutathione, 2 mM adenosine-5'-triphosphate, 200 μg of the enzyme, and the test compound (dissolved in 4 μL DMSO) in a final volume of 200 μL. Preincubation of enzyme with the test compound was performed at 30 °C for 5 min. Reaction was started by adding 16 μM [1-^{14}C]arachidonic acid (6.29 kBq/5 μL of ethanol), performed with shaking at 30 °C for 30 min, and terminated by adding 50 μL of 0.2 N citric acid. Arachidonic acid and its metabolites were extracted with 1.5 mL of ethyl acetate. The ethyl acetate layer (1 mL) was dried under a nitrogen gas stream and spotted on a silica gel plate. Thin-layer chromatography was carried out with a solvent system of diethyl ether-petroleum ether-acetic acid (85/15/0.1). Radioactivity on the plate was monitored with the use of a Packard radiochromatogram scanner. For quantitative determination of the enzyme activity, the silica gel zones corresponding to authentic 5-hydroxyeicosatetraenoic acid (5-HETE) were scraped into scintillation vials. Radioactivity was determined by a Packard liquid-scintillation counter. The enzyme activity was expressed in terms of the amount of 5-HETE synthesized for 30 min.

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Registry No. **7a**, 100480-35-1; **7b**, 100480-15-7; **7c**, 100480-16-8; **7d**, 100480-17-9; **7e**, 100480-19-1; **7f**, 100480-20-4; **4g**, 100480-21-5; **7h**, 100505-37-1; **7i**, 100480-24-8; **7j**, 100480-81-7; **7k**, 100480-40-8; 7l, 100480-23-7; **15a**, 83857-82-3; **17a**, 100479-81-0; **17b**, 125714-62-7; **18**, 100479-96-7; **19**, 125714-63-8; **20a**, 125714-63-8; **20b**, 125714-64-9; **24**, 100480-05-5; **25**, 56539-25-4; **26**, 100480-67-9; **27a**, 950-99-2; **27b**, 79907-49-6; **27c**, 56305-04-5; EtCOCl, 79-03-8; PrCOCl, 141-75-3; BuCOCl, 638-29-9; $C_6H_{13}COCl$, 2528-61-2; $C_7H_{15}COCl$, 111-64-8; $C_{19}H_{39}COCl$, 40140-09-8; methyl succinyl chloride, 1490-25-1; *tert*-butylhydroquinone, 1948-33-0; 5,5'-bis(1,1-dimethylethyl)-2,2'-dithiobishydroquinone, 125714-65-0; 5,5'-bis(1,1-dimethylethyl)-2,2'-trithiobishydroquinone, 125714-66-1.

Supplementary Material Available: A table of hypolipidemic activity of 1,3-benzoxathioles along with compound **2** are available (1 page). Ordering information is given on any current masthead page.

Specific Bradycardic Agents. 1. Chemistry, Pharmacology, and Structure-Activity Relationships of Substituted Benzazepinones, a New Class of Compounds Exerting Antiischemic Properties

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Structural modification of the calcium-antagonist verapamil (1) by replacement of the lipophilic α-isopropylacetonitrile moiety by various heterocyclic ring systems has led to a new class of cardiovascular compounds which are characterized by a specific bradycardic activity. These agents reduce heart rate without binding to classical calcium channels or β-adrenoceptors, interacting instead specifically with structures at the sino atrial node. Therefore they have also been termed sinus node inhibitors. The prototype falipamil (2) has been submitted to further optimization mainly by manipulation of the phthalimidine moiety. This has resulted in a second generation of specific bradycardic agents with increased potency and selectively and prolonged duration of action represented by the benzazepinone-derivative UL-FS 49 (4). Structure-activity relationships within this novel class of compounds have revealed a marked dependence of activity on the substitution pattern of the aromatic rings, the nature of the central nitrogen atom, and the length of the connecting alkyl chains. The crucial role of the benzazepinone ring for bradycardic activity can be best explained by its special impact on the overall molecular conformation.

Ischemic heart disease is characterized by an imbalance between myocardial substrate supply and demand. This imbalance may result in ischemic pain, myocardial dysfunction, or tissue necrosis. Heart rate is a major determinant of myocardial energy demand.[1] Thus, drug-induced bradycardia would be expected to reduce myocardial oxygen consumption.[2] In addition, bradycardia may increase blood flow to the subendocardial layers of the myocardium, which are predominantly perfused during diastole.[3,4] Two classes of pharmacological agents which are frequently used in the treatment of ischemic heart disease induce bradycardia. These include the β-adrenoceptor antagonists[5] and calcium channel blockers, the most prominent being verapamil and diltiazem.[6] However, most β-blockers and calcium channel blockers not only reduce heart rate but also reduce myocardial contractile force. In addition, the latter agents are capable of reducing the coronary perfusion pressure, which may result in a decrease in ischemic coronary flow and myocardial perfusion.

Because of these potential drawbacks of β-blockers and calcium channel blockers, a synthesis program was initiated aimed at compounds which selectively reduce heart rate

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